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Lung responses in murine models of experimental asthma: Value of house dust mite over ovalbumin sensitization

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ABSTRACT

Ovalbumin (OVA) sensitization has limitations in modelling asthma. Thus, we examined the value of allergic sensitization using a purified natural allergen, house dust mite (HDM), over the sensitization performed with OVA. Mice were sham-treated, or sensitized with OVA- or HDM with identical chronology. Airway resistance, tissue damping and elastance were assessed under control conditions and after challenging the animals with methacholine (MCh) and the specific allergen. Inflammatory profile of the bronchoalveolar lavage fluid was characterized and lung histology was performed. While no difference in the lung responsiveness to the specific allergen was noted, hyperresponsiveness to MCh was observed only in the HDM-sensitized animals in the lung peripheral parameters. Lung inflammation differed between the models, but excessive bronchial smooth muscle remodelling occurred only with OVA. In conclusion, we demonstrate that a purified natural allergen offers a more relevant murine model of human allergic asthma by expressing the key features of this chronic inflammatory disease both in the lung function and structure.

1. Introduction

Increasing exposure to environmental allergens and pollution contribute to the dramatic rise in the incidence of chronic lung diseases such as asthma, which is a major public health concern (ELF, 2009; WHO, 2008). One of the main features of asthma is chronic airway inflammation with subsequent bronchial hyperresponsiveness to different stimuli (Bosse, 2014; Brutsche et al., 2006; Mannino and Buist, 2007). Various animal models contributed greatly to a better understanding of the underlying pathophysiological mechanisms and to the characterization of the complex interactions between allergic, neurologic and immunological pathways (Gern, 2008).

In the last decades, airway sensitization to ovalbumin (OVA) was considered as the experimental model of reference to investigate the pathogenesis of allergic lung diseases (Bayat et al., 2009; Habre et al., 2008). However, the value of OVA in these models to mimic human asthma has been challenged due to the lack of key mechanisms such as eosinophil migration, sustained chronic airway inflammation and the development of allergen tolerance leading to desensitization (DiGiovanni et al., 2009; Fattouh et al., 2005; Johnson et al., 2004; Swirski et al., 2002). Another important limitation of OVA exposure is related to the site of lung mechanical response. This is limited to the central conducting airways after an exposure to a nonspecific constrictor stimuli, such as methacholine or histamine (Bayat et al., 2009), despite the involvement of small airways in the lung function deteriorations during asthma exacerbations (Bjermer, 2014; Contoli et al., 2012).

Recently, there has been an increased interest towards using natural airborne allergens in the animal models of allergic respiratory diseases. Among them, dermatophagoides pteronissinus (*D. pter*) is the most widespread dust mite with Der p1 being one of the major proteins implicated in the allergic process induced by *D. Pter* (Tournoy et al., 2000; Tovey et al., 1981). It has been identified as having a cysteine protease activity capable of inducing epithelial desquamation, inflammatory cytokines release and allergens transport facilitation (Kauffman et al., 2006). It stimulates pulmonary inflammation, IgE humoral response (Gough et al., 2003; Kikuchi et al., 2006) and Th2 cell response (Comoy et al., 1998). Therefore, sensitization to house dust mite (HDM) appears the most clinically relevant asthma model in mice and is gaining interest in experimental research (Kikuchi et al.,

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2006). However, to date, the contribution of the conducting airways and the lung periphery to the lung mechanical responses following exogenous constrictor stimuli have not been compared between murine models of OVA and HDM sensitization. Additionally, such characterization is still missing following provocation with the specific allergen, which is of clinical relevance since asthma exacerbation occurs essentially following repeated airborne allergen exposures.

Therefore, we aimed at comparing the responses in the central conducting airways as well as in the lung periphery between mice sensitized and exposed to OVA with those sensitized and exposed to Der p1. Moreover, we aimed at investigating the differences between the 2 sensitization regimens following a lung constriction induced by non-specific cholinergic stimuli and during the early phase of allergic response.

2. Materials and methods

2.1. Animals

All experiments and procedures were conducted under the approval from the Swiss animal welfare committee (Geneva Cantonal Veterinary Office, registration number 1043/3924/2). Forty-four 10 week-old (22.0 ± 0.2 g) female BALB/cAnNRj mice purchased from Janvier Labs (St Berthevin, France) were involved in the study. Female BALB/c mice were chosen because of the most susceptible gender and strains to respiratory allergies including for HDM and OVA (Berndt et al., 2011; Blacquiere et al., 2010; Leme et al., 2010; Melgert et al., 2005).

2.2. Sensitization to allergens

Lyophilized total protein extracts of *D. pter* with known concentration of Der p1 were obtained from Stallergenes (Antony, France). Lyophilized ovalbumin from chicken egg white was purchased from SigmaAldrich (Buchs, Swizerland). Both substances were suspended in normal saline. Mice were sensitized to HDM with a protocol adapted from Tourdot et al. (2011). For an appropriate comparison, sensitizations with OVA and HDM were performed according to the same chronology. For both allergens, the procedure was initiated with two intraperitoneal injections of OVA (50 µg/200 µL) or HDM (dose equivalent to 10 µg Der p1 conveyed in 200 µL) supplemented with Al $(OH)_3$ as an adjuvant (2 mg) (Fig. 1A). 30-min long aerosol exposures to the allergens were then performed between days 21–25 with OVA (1 mg/mL) and HDM (dose equivalent to 0.1 mg/mL of Der p1), respectively. The same injections and aerosol exposures were carried out for the control group of mice with normal saline (SAL).

2.3. Anaesthesia and surgical preparation

Mice were anesthetized on day 28 or 29 with 5% isoflurane in a box until reflexes disappeared. Then a subcutaneous injection of a mixture of metomidate (Syndel Laboratories Ltd., Canada; 60 mg/kg) and fentanyl (60 µg/kg) was administered. After 5 min, a local anaesthesia by subcutaneous injection of 0.5% xylocaine (0.5 mL) was provided and a tracheostomy was performed. Mice were tracheotomised and mechanically ventilated with Flexivent system (Emka Technologies, Falls Church, VA, USA) in volume- controlled mode (tidal volume 8 mL/kg, 180 breaths/min, I:E 1/1.5, positive end-expiratory pressure (PEEP) 3 cmH_2O, FiO_2 50%). The mice were paralyzed thereafter with an intraperitoneal injection of muscle relaxant (5 mg/kg rocuronium bromide) to inhibit spontaneous breathing movements. This injection was repeated as needed (onset of respiratory movements during measurements). Similarly, the anaesthesia was maintained by additional intraperitoneal boluses of the anaesthetics at half doses of the concentrations used for the induction. Body temperature was maintained between 38 and 39 °C by using a heating mat and controlled rectally by a thermal sensor. ECG was continuously monitored by means of needle electrodes and PowerLab data acquisition system (ADinstrument, Dunedin, New Zealand).

2.4. Challenges and protocol groups

Mice were assigned into one or the other of the 7 protocol groups (Fig. 1B). Respiratory function was assessed in the anesthetized and ventilated animals by using the Flexivent system. In the mice challenged with the allergens, measurements were made during the baseline conditions and for 30 min following a single challenge of aerosolized OVA (1 mg/mL for 40s) in the Groups SAL/OVA (n = 6) and OVA/OVA (n = 8) or *D. pter* (0.1 mg/mL for 40 s) in the Groups SAL/HDM (n = 5) and HDM/HDM (n = 7). Measurements were made each 30 s until 2 min, then each 1 min for the following 3 min, every 2 min until 15 min followed by 20 and 30 min recording. In the other mice enrolled in groups SAL/MCh (n = 6), OVA/MCh (n = 8) and HDM/MCh (n = 6), basal respiratory mechanical measurements were repeated during an aerosolized challenge with a non-specific cholinergic constrictor agonist, methacholine (MCh) administered at increasing dose of 0.78, 1.56, 3.125, 6.25, 12.5 mg/mL (for 10 s each).

2.5. Measurement of respiratory mechanics

Lung volume history was standardized while inspiratory capacity (IC) was measured during the first deep inflation manoeuvre (slow inflation from PEEP to 30 cm H_2O with 3 s breath hold). Forced oscillatory respiratory mechanics were measured with Flexivent system. Respiratory system input impedance (Zrs) was derived from the signals detected by the ventilator piston volume displacement and cylinder pressure during 8 s oscillatory volume perturbations with 17 prime frequencies ranging from 0.5 and to 19.75 Hz. The impedance data were fitted with a model comprising an airway resistance (Raw) and inertance in series with a constant-phase model incorporating tissue damping (G) and elastance (H) (Hantos et al., 1992). Respiratory tissue hysteresivity (η) was calculated as G/H (Fredberg and Stamenovic, 1989).

2.6. Assessment of lung inflammation

At the end of the experiment, the animal was disconnected from the ventilator. Pre-warmed (38 °C) PBS was instilled via the endotracheal tube with a syringe containing a volume of 30 µL/g body weight (total lung volume of BALB/c mice (Thiesse, 2009)). Three gentle wash-in and wash-out maneuvers were made with the syringe to collect the bronchoalveolar lavage fluid (BALF); thereafter the animal was immediately euthanized. The BALF was centrifuged at 2000 rpm for 5 min at 5 °C and supernatant sampled and stored at −20 °C until use. The cell pellet was resuspended in BSA 1% in PBS, further dropped onto slides and centrifuged at 750 rpm for 7 min by cytospin. Then slides were fixed and stained with May Grünwald Giemsa for differential cell counting. Then stained slides were scanned by Mirax and the cells were counted by using an image acquisition software (Panoramic viewer, 3DHISTECH Ltd, Budapest, Hungary). Since the distribution of the cells was inhomogeneous, the cells were counted within rectangles with an edge length equivalent to the radius of the circular cytospin. The number of cells was normalized to the surface area of the rectangles.

2.7. Cytokine essays

The BALF supernatant and the blood serum were used to assess inflammatory cytokines. Blood samples were centrifuged at 4500 rpm for 3 min to collect serum, which was diluted 4× before the assays. Immuno assay with an appropriate software (Bio-Plex Manager™ Multiplex Reader) was used to measure fluorescence in the multiplex kit (Bio-Plex Pro™ Mouse Cytokine Th1/Th2 Assay #M6000003J7). This kit allowed the measurement of eight mice cytokines GM-CSF, IFN-γ, IL-

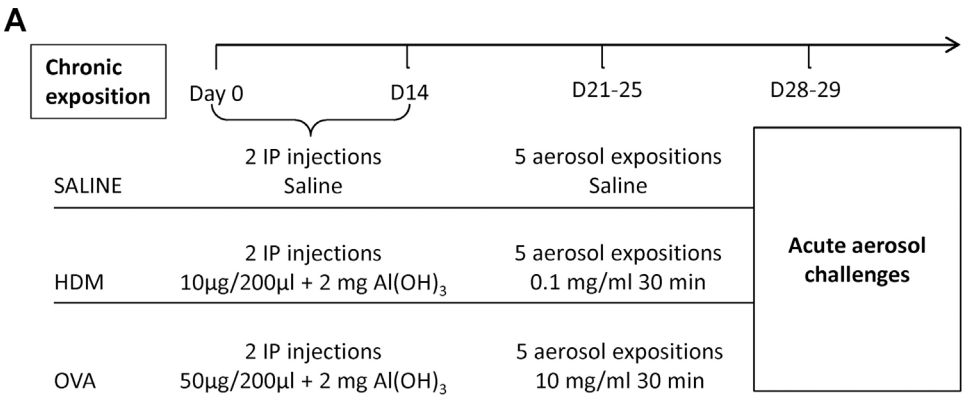

Fig. 1. A: Scheme of the sensitization procedure. B: summary of the protocol groups exposed chronically to different allergens challenged acutely with a non-specific constrictor stimulus (methacholine) or to the specific allergen (ovalbumin or house dust mite).

B

	Chronic exposition			Acute challenge		
group	Saline	Ovalbumin	House Dust Mite	Methacholine	Ovalbumin	House Dust Mite
SAL/MCh	+			+		
SAL/OVA	+				+	
SAL/HDM	+					+
OVA/MCh		+		+		
OVA/OVA		+			+	
HDM/MCh			+	+		
HDM/HDM			+			+

2, IL-4, IL-5, IL-10, IL-12 (p70), TNF-α).

2.8. Lung histology

At the end of the experiment, the lungs were excised, rinsed with PBS and filled with 4% formaldehyde under a pressure of 20 cmH_2O. The fixed lungs were embedded into paraffin wax and 10 µm sections were made, mounted on slides and stained with haematoxylin and eosin to determine cellular infiltrates.

5 µm thick lung tissue sections were made from the formalin-fixed, paraffin-embedded lung tissues and were analysed by immunohistochemistry using anti-alpha-smooth muscle actin (αSMA, anti-AML, Dako, #M0851, 70 mg/mL) with the Ventana Discovery automated staining system (Ventana Medical Systems, Tucson, AZ, USA). For anti-alpha smooth muscle actin antibodies, no antigen retrieval pre-treatment was required. After automatic deparaffinization, slides were incubated 30 min at 37 °C with primary antibodies diluted at 1/300 in antibody solution from Dako Schweiz GmbH (Switzerland, ref. S2022). Then secondary antibodies were applied at dilution 1/250 (anti-mouse IgG1 + IgG2a + IgG3, abcam, ab133469, 2.03 mg/mL). Detection of secondary antibodies was carried out using the rabbit OmniMap kit (Ventana Medical Systems, Tucson, AZ, USA), based on conversion of diaminobenzidine to a dye with multimeric horseradish peroxidase.

The paraffin-embedded lung tissue sections were also stained with Masson trichrome (Sigma-Aldrich®, Switzerland) to determine the relative proportion of sub-epithelial collagen deposition. These sections were analysed by using the Developer XD2 image analysis software (Definiens AG, Munich, Germany).

2.9. Statistical analyses

Considering the variability in the primary outcome, Raw, and based on our previous experiment with similar methodology (Petak et al., 2001), the study was planned in order to detect at least 40% difference between groups by using 80% power. Standard errors of the means (SEM) are reported. Normal distribution was verified with the Shapiro-Wilk test. In case of lack of normality, logarithmic transformation was performed before applying parametric tests. The grouped data of BALF and respiratory mechanics were tested with two-way analyses of variances (ANOVA) with Holm-Sidak post-hoc tests. Total cell counts in the BALF, IC, histological and cytokine data were evaluated by using one-way ANOVA tests. The statistical tests were performed with SigmaPlot (Version 12.5, Systat Software, Inc.) and Prism (version 6, GraphPad Software Inc.).

3. Results

3.1. Basal respiratory mechanical parameters

Table 1 summarizes the absolute values of the airway and

Table 1
Baseline values (mean ± SEM) of respiratory mechanical parameters in the protocol groups including mice exposed to an allergen (SAL/OVA, OVA/OVA, SAL/HDM and HDM/HDM) or a metchacholine as a non-specific contrictor stimuli (SAL/MCh, OVA/MCh, HDM/MCh).

	Raw (cmH_2O s/mL)	G (cmH_2O/mL)	H (cmH_2O/mL)	η
SAL/OVA	0.23 ± 0.01	4.18 ± 0.10	15.56 ± 0.22	0.25 ± 0.01
OVA/OVA	0.26 ± 0.01	4.03 ± 0.15	16.58 ± 0.50	0.25 ± 0.01
SAL/HDM	0.26 ± 0.02	3.74 ± 0.07	15.05 ± 0.50	0.24 ± 0.01
HDM/HDM	0.29 ± 0.02	3.83 ± 0.11	16.53 ± 0.69	0.27 ± 0.00
SAL/MCh	0.28 ± 0.03	3.66 ± 0.10	14.51 ± 0.58	0.24 ± 0.01
OVA/MCh	0.25 ± 0.02	3.99 ± 0.08	15.88 ± 0.48	0.25 ± 0.00
HDM/MCh	0.30 ± 0.01	3.83 ± 0.13	16.35 ± 0.75	0.23 ± 0.01

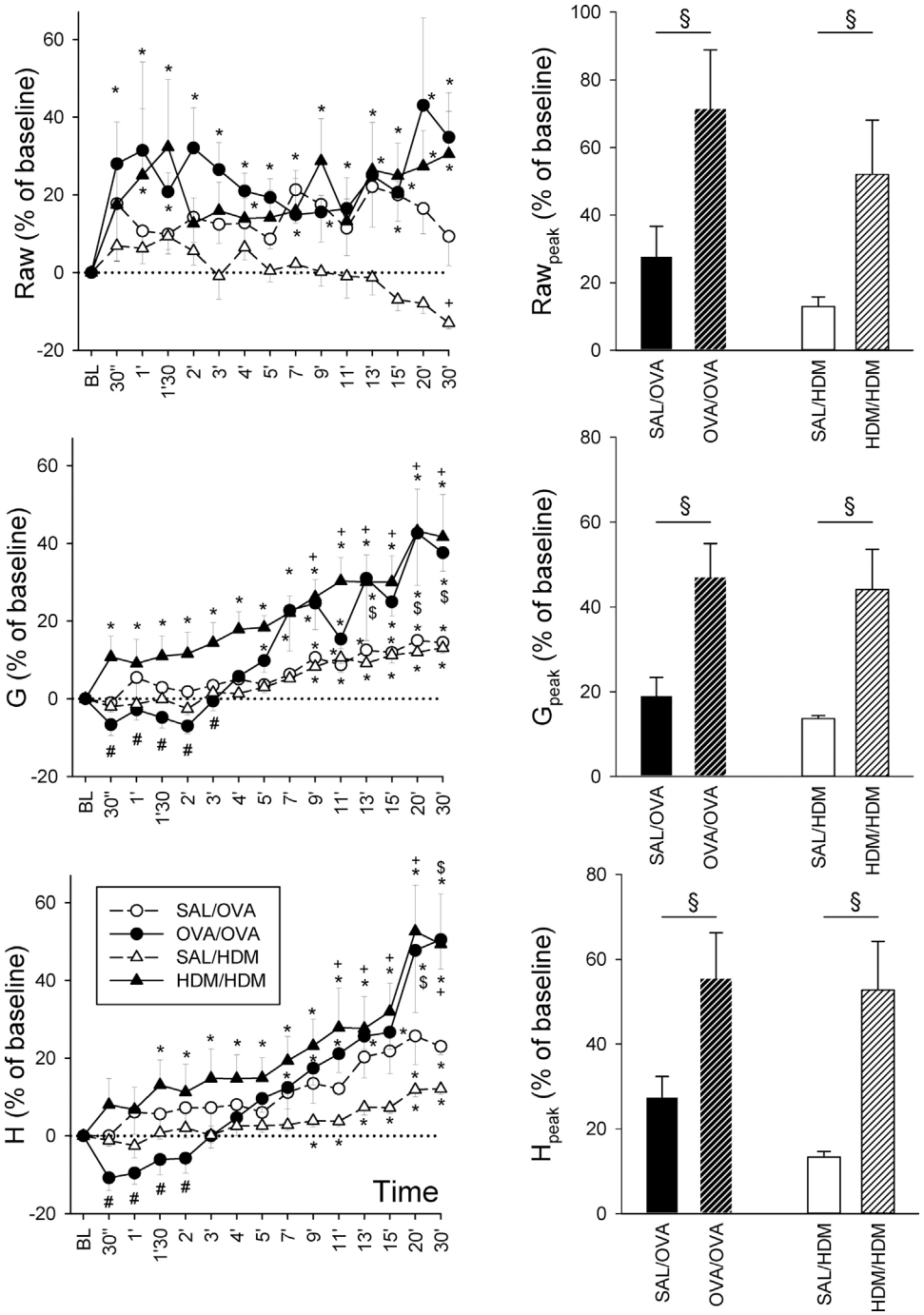

Fig. 2. Left panels depict the temporal changes (mean ± SEM) in the airway (airway resistance: Raw) and tissue mechanics (tissue damping: G, tissue elastance: H), while right panels show the group mean of the peak responses (mean ± SEM) following exposures to the specific allergens ovalbumin (OVA) or house dust mite (HDM). SAL/OVA (n = 6): sham-sensitized mice exposed to OVA, OVA/OVA (n = 8): OVA-sensitized mice exposed to OVA, SAL/HDM (n = 5): sham-sensitized mice exposed to HDM, HDM/HDM (n = 7): HDM-sensitized mice exposed to HDM. *: $p < 0.05$ vs. baseline (BL) within a group, #: $p < 0.05$ OVA/OVA vs. HDM/HDM, $: $p < 0.05$ SAL/OVA vs. OVA/OVA, +: $p < 0.05$ SAL/HDM vs. HDM/HDM, §: $p < 0.05$ between peak responses (Two-way repeated measures ANOVA).

respiratory tissue parameters under the baseline conditions. No statistically significant differences were detected in any of these variables between the protocol groups. The IC exhibited significant decreases in mice sensitized with both OVA (0.867 ± 0.02 mL, $p < 0.001$) and HDM (0.951 ± 0.02 mL, $p < 0.01$), as compared to control animals (1.06 ± 0.03 mL).

3.2. Lung responses to the specific allergens

Fig. 2 displays on the left panels the time course of the airway and respiratory tissue parameters following challenges with the specific allergens. The group means of the peak responses obtained in each individual mouse are also demonstrated on the right panels. Immediate increases in Raw were observed in both groups of sensitized mice ($p < 0.05$), which were sustained throughout the 30-min study period. Parameters reflecting the lung peripheral changes exhibited similar changes with marked differences in the temporal dynamics: OVA challenge led to delayed elevations in G ($p < 0.001$) and H ($p < 0.001$), whereas HDM resulted in immediate responses in these indices ($p < 0.001$ for both).

In accordance with these time courses, the peak airway responses in the HDM- and OVA-exposed animals were manifested in significant increases following the allergen exposure ($p < 0.05$ for both) when compared to the non-sensitized controls. Similarly, both allergens increased G and H significantly with peak elevations being greater in the allergen-sensitized mice than their corresponding controls ($p < 0.01$ and $p < 0.02$). The proportional increases in G and H resulted in a constant η in mice challenged with OVA or HDM, with no significant

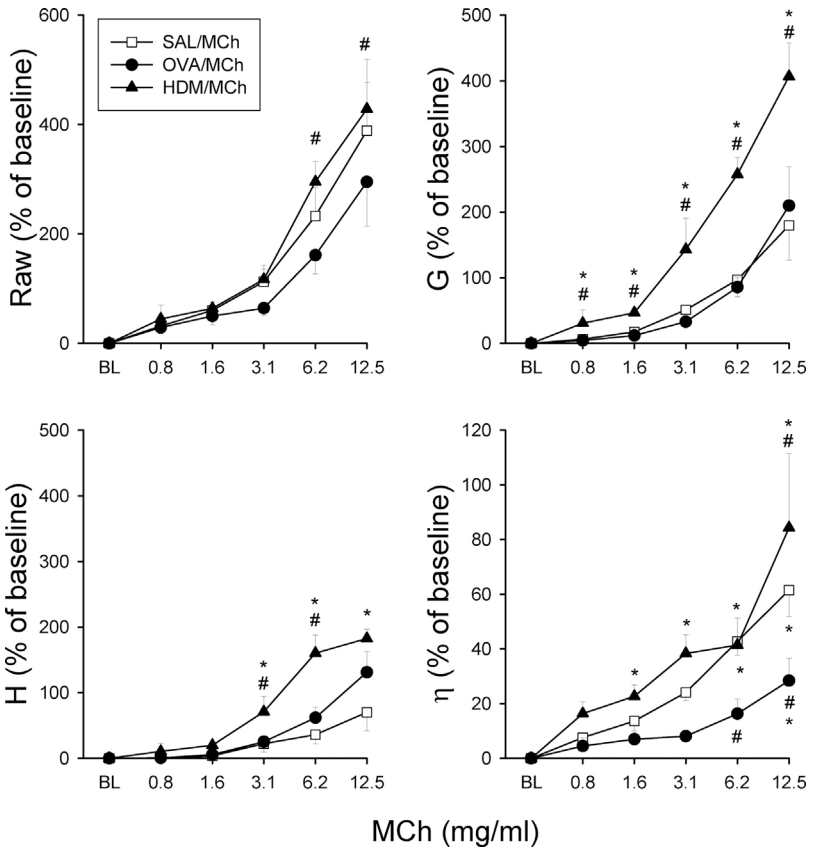

Fig. 3. Dose response curves (mean ± SEM) in airway resistance (Raw), tissue damping (G), tissue elastance (H) and hysteresivity (η) to aerosolized methacholine (MCh) in sham-sensitized mice (SAL/MCh, n = 6) and in mice sensitized with ovalbumin (OVA/MCh, n = 8) or house dust mite (HDM/MCh, n = 6). *: $p < 0.05$ vs. baseline (BL) within a group, #: $p < 0.05$ between OVA/MCh and HDM/MCh (Two-way repeated measures ANOVA).

difference between the protocol groups.

3.3. Lung responsiveness to a nonspecific cholinergic challenge

Fig. 3 depicts the dose response curves for the respiratory mechanical parameters following administrations of increasing doses of an aerosolized nonspecific constrictor agonist, MCh. The allergen sensitized groups responded differently to MCh. While there was no evidence for a difference between the OVA/MCh and the control SAL/MCh groups in Raw, G and H, HDM-sensitized mice exhibited exaggerated responses in Raw compared to those receiving OVA ($p < 0.05$). Moreover, MCh induced significantly greater responses in the parameters G and H in the HDM-sensitized mice compared to OVA-sensitized and to control mice ($p < 0.02$ and $p < 0.05$, respectively). The excessive increases in G over those on H were reflected in the elevated η in the HDM exposed mice following administration of the highest dose of MCh ($p < 0.01$).

3.4. Lung inflammation

Fig. 4 shows the total leucocytes count in the bronchoalveolar lavage fluid and the relative proportion of the inflammatory cell components. The total amount of inflammatory cells was significantly elevated in the OVA and HDM sensitized mice relative to their controls ($p < 0.001$ and $p < 0.01$, respectively). While macrophages were consistently dominant in all control groups, eosinophil influx was detected in both sensitized groups, with a more apparent eosinophilia in the HDM/HDM group ($p < 0.001$). These changes were associated with significant decreases in the relative proportion of macrophages in the OVA/OVA and HDM/HDM groups compared to their corresponding controls ($p < 0.001$ for both).

3.5. BALF cytokine profile

The cytokine concentrations in the BALF supernatant are shown in Fig. 5. The allergen sensitization with OVA and HDM led to marked differences in the BALF cytokine production. OVA sensitization induced changes in the BALF cytokine profile with increased IL-4 ($p < 0.03$), and decreased IL-2 ($p < 0.03$), IL-10 ($p < 0.03$), IL-12 ($p < 0.001$) and GM-CSF ($p < 0.001$), and no detectable change in IL-5 and IFN-γ. Sensitization with HDM significantly increased secretion of IL-4 ($p < 0.001$), and decreased release of IL-2 ($p < 0.02$), IL-12 ($p < 0.001$) GM-CSF ($p < 0.001$) and TNF-α ($p < 0.001$), with no detectable change in IL-5, IL-10 and IFN-γ. Compared to the OVA group, BALF from HDM-sensitized mice contained higher concentrations of IL-4 ($p < 0.03$) IL-10 ($p < 0.05$) and IF-γ ($p < 0.05$).

3.6. Histological evaluation

Fig. 6 summarizes the histological findings in the animals included in the protocol groups. Epithelial thickness was significantly elevated in both groups of mice sensitized with OVA and HDM ($p < 0.001$ for both). Over-expression of α-SMA positive cells was detected in both sensitized groups ($p < 0.001$ and $p < 0.05$ for OVA and HDM, respectively) with a significantly higher over-expression in the mice sensitized with OVA ($p < 0.001$). Conversely, sub-epithelial collagen deposition was significantly enhanced in the HDM group both in comparison with the control mice ($p < 0.05$) and those sensitized with OVA ($p < 0.001$).

4. Discussion

The results of the present study revealed fundamental differences between two different models of allergen sensitization using a natural allergen extract (OVA) and a purified natural allergen (HDM). Hyper responsiveness to a cholinergic non-specific constrictor stimulus and

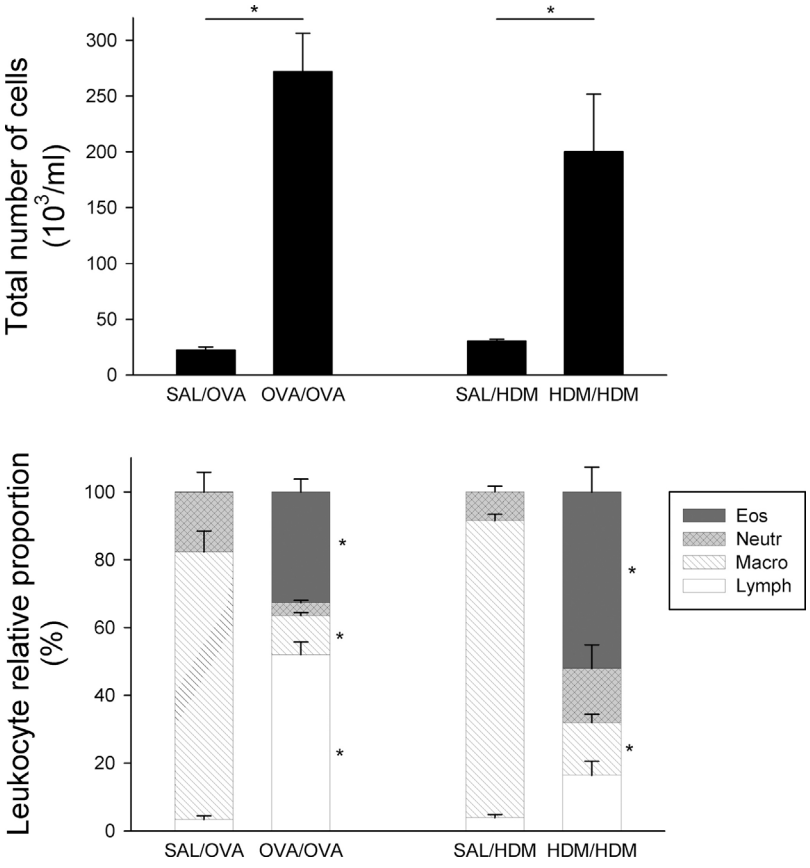

Fig. 4. Total number of leukocytes in the bronchoalveolar lavage fluid (top) and the relative differential cell counts for the mice enrolled in the protocol groups. SAL/OVA (n = 6): sham-sensitized mice exposed to OVA, OVA/OVA (n = 8): OVA-sensitized mice exposed to OVA, SAL/HDM (n = 5): sham-sensitized mice exposed to HDM, HDM/HDM (n = 7): HDM-sensitized mice exposed to HDM. *: $p < 0.05$ vs. the corresponding sham-sensitized (Two-way ANOVA).

alterations in the BALF cellular and cytokine profiles observed in the HDM model appeared more relevant to the human asthma phenotype. Conversely, both models exhibited similar airway and tissue responsiveness to the specific allergen. Histological investigations evidenced comparable epithelial thickening, with increased bronchial smooth muscle remodelling in OVA-sensitized mice as reflected by the overexpression of αSMA antibodies.

4.1. Lung responsiveness to a nonspecific cholinergic challenge

In an attempt to characterize the bronchial hyperreactivity following OVA and HDM sensitization, the mice were challenged with incremental doses of inhaled methacholine. This cholinergic stimulation led to a parallel increase in Raw in all groups of animals with no evidence for a difference as compared with the saline exposed mice. The mechanism contributing to this finding may be related to the potential contamination of the commercial allergens with lipopolysaccharide, which has been shown to blunt murine immunologic responses and prohibits the development of BHR (Watanabe et al., 2003). However, comparing OVA- and HDM-sensitized mice revealed significantly greater central airway responses to MCh in the latter model.

As the lung peripheral responses are concerned, a clear-cut finding was observed in the lung tissue parameters with a significant response in the mice sensitized with HDM. These results, along with significant decrease in IC, suggest that the eosinophilic inflammation following HDM exposure is localized preferably in the parenchyma rather than in the central airways (Layachi et al., 2013). Interestingly, the hysteresivity; G/H, increased dose-dependently during methacholine challenge, which suggests that the alteration of tissue resistance following MCh-induced constriction is also due to increased airway inhomogeneity (Lutchen et al., 1996). The primary involvement of the lung periphery following MCh challenges agree with the results of previous studies in HDM-sensitized mice (Li et al., 2014; Phan et al., 2016).

The development of BHR following OVA-sensitization is a subject of controversy with some papers demonstrating airway hyperresponsiveness to a non-specific constrictor challenge (Barlow et al., 2011; Kaufman et al., 2011; Wang et al., 2011), while others failed to detect increased airway narrowing following a similar sensitization regimen with OVA (Bates et al., 2008; Card et al., 2010; Yiamouyiannis et al., 1999). One possible explanation for these conflicting results is the potential desensitization of the airways following repeated OVA exposures (DiGiovanni et al., 2009; Fattouh et al., 2005; Ruiz Schutz et al., 2009; Swirski et al., 2002; Yiamouyiannis et al., 1999). The existence of this potential tolerance to chronic exposure to OVA can be anticipated from the altered cytokine profile in OVA-sensitized mice. The tendency for a decrease in IL-5 and IL-10, along with lower eosinophilia and lack of BHR strongly suggest progression towards a tolerance process. These findings indicate essential differences in the airway smooth muscle responsiveness to a cholinergic challenge, with better suitability of the HDM model to investigate this important feature of human asthma.

4.2. Lung responses to the specific allergens

Despite the frequent investigations of OVA, assessment for airways and tissue mechanics following purified natural allergen (such as HDM) remains scarce (Li et al., 2014), and is limited to a short time follow-up in the sub-acute phase (< 3 min). In the present study we extended the observation windows to characterize airway and respiratory tissue mechanical responses to 30 min. The results revealed remarkable differences in the temporal dynamics of the peripheral lung responses between mice exposed to OVA and those sensitized with HDM, deteriorations of tissue mechanics starting as early as 30 s in the former, whereas only after 5 min in the latter case.

While the reason for this finding is not completely clear, the distinct lung inflammatory profile may explain the differences between the two

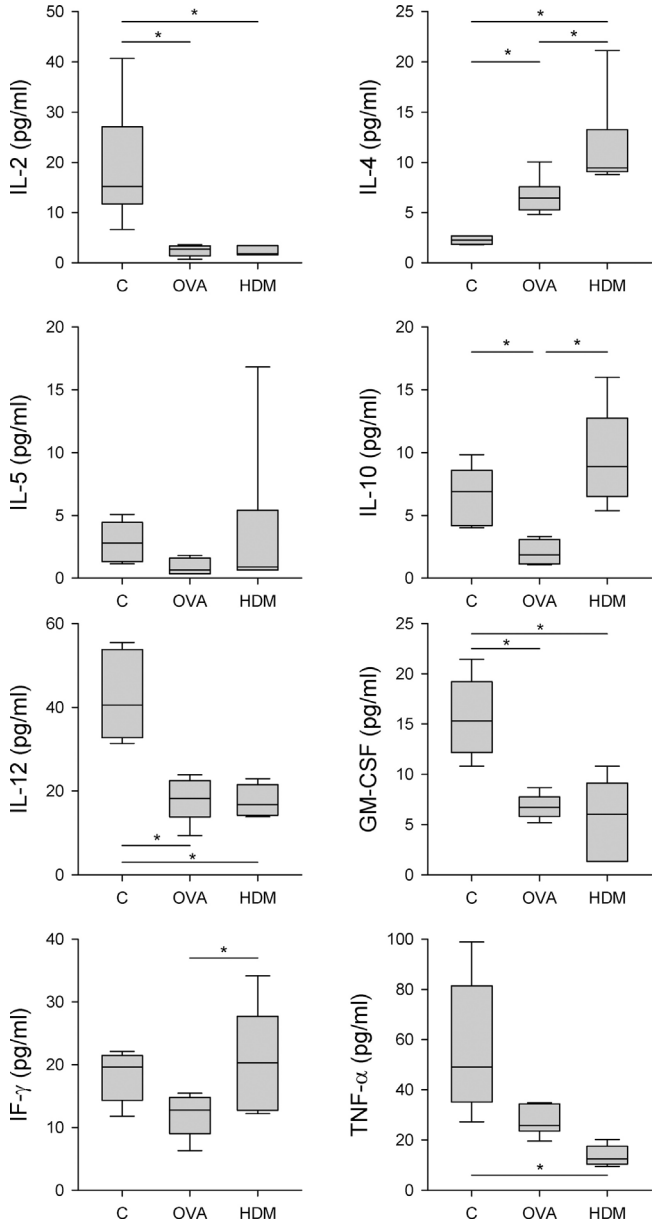

Fig. 5. Cytokine concentrations in the bronchoalveolar lavage fluid in sham-sensitized mice (C, n = 6) and in mice sensitized with ovalbumin (OVA, n = 8) or house dust mite (HDM, n = 7). *: $p < 0.05$ between protocol groups (one-way ANOVA).

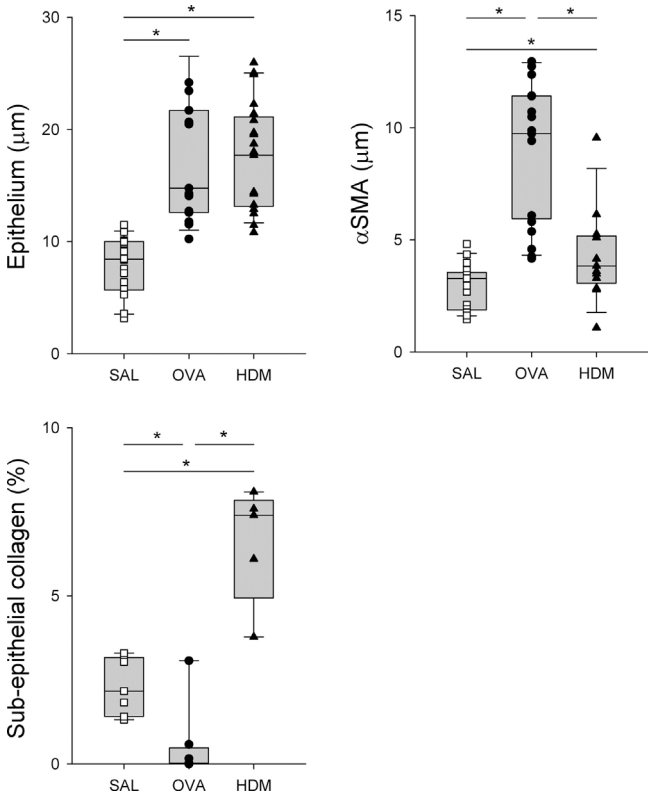

Fig. 6. Epithelial thickness (top left), thickness of smooth muscle contractile elements (αSMA, top right), and relative amount of sub-epithelial collagen (bottom) in sham-sensitized mice (C, samples based on n = 6) and in mice sensitized with ovalbumin (OVA, samples based on n = 8) or house dust mite (HDM, samples based on n = 7). *: $p < 0.05$ between protocol groups (one-way ANOVA).

sensitization modes. The proportionally greater influx of eosinophils and neutrophils in the BALF (Fig. 4), with increased specific allergic inflammatory cytokines IL-4 and IL-10, as well as reduced anti-inflammatory cytokines IL-2 and IL-12 in HDM indicates more intense inflammatory response in the peripheral airways (Fig. 5) (Tourdot et al., 2011). This inflammatory profile may have contributed to a rapid and sustained mast cell degranulation in the HDM-sensitized mice. Since η remained constant, as G and H changed together, we can hypothesize that allergen exposure led to either changes in the intrinsic viscoelastic properties of the lung parenchyma, and/or a uniform closure of a terminal alveolar unit due to the uneven deposition of the constrictor agonist.

As concerns the lack of increase in TNF-α in the sensitized animals, the timing of the experiments from the allergen challenge may have played a role, since the concentration of this cytokine has been shown to decline rapidly by 24 h after the allergen exposure (Kim et al., 2006). Thus, TNF-α may have been at the nadir level by the time of our experiment, which was performed 72 h after the allergen exposure, while IL-4 were still overexpressed. Moreover, the reason for the lack of increase in GM-CSF may be explained by the dependency of its expression by the clinical subtype (Kuo et al., 2017). It is also possible that in the current models, the decreased concentration of macrophages in the sensitized groups may have further contributed to the diminished GM-CSF protein concentration measured in the BALF.

4.3. Allergic asthma models: OVA versus HDM

In the present study, the sensitization procedure followed similar chronological administration with the 2 allergens. Previous reports highlighted the importance of protocol designs both in their chronology and dose of administration, for the development of a model of bronchial hyperreactivity (Chang et al., 2005; Petak et al., 2010). The discrepancy between OVA and HDM seems to be related to the nature of the allergen itself or to the repeated administration, which may have led to desensitization as discussed above (Swirski et al., 2002; Yeh et al., 2015). However, we cannot rule out that these two allergens may have different sensitization procedures required to reach their optimal effect. The involvement of time and regularity of allergen exposure requires further investigations.

The difference between the two allergen models was also revealed in an earlier study following chronic intranasal OVA or HDM exposure with the same chronology in BALB/c mice (Johnson et al., 2004). A difference in the respiratory patterns, assessed by unrestrained body plethysmography, between HDM-sensitized and OVA-exposed mice was associated with the presence of lung inflammation only in the HDM-sensitized group.

Applying different optimized protocols for sensitization with OVA and HDM also demonstrated that the latter was more efficient in inducing an experimental model of asthma (DiGiovanni et al., 2009). In

line with our findings, the use of HDM was more effective in inducing lung inflammation and increase reactivity to MCh which was not related to overexpression of contractile smooth muscle cells but rather to a Th2/Th1 imbalance (DiGiovanni et al., 2009; Li et al., 2014; Piyadasa et al., 2016). Moreover, mice sensitized with HDM were the only one that exhibited pro-fibrotic lung tissue remodelling, which is a hallmark of asthma (Locke et al., 2007). This finding agrees with previous results demonstrating the importance of the combination of airway inflammation, sub-epithelial fibrosis and airway hyperresponsiveness in the pathogenesis of asthma (Royce et al., 2014). This structural impairment is in line with the excessive increases observed in the lung function parameters reflecting the lung periphery (G and H).

4.4. Summary and conclusions

The present study related lung mechanical responses to cholinergic and allergen stimuli, the inflammatory profile and airway remodelling in murine models of asthma. Our findings revealed that sensitization with HDM led to a more specific airway inflammation expressed in the augmentation of eosinophils and a Th1/Th2 imbalance. This inflammatory response was associated with enhanced lung peripheral response to a non-specific cholinergic constrictor stimulus, which was not expressed in the animals sensitized with OVA. These results demonstrate that sensitization with a purified natural allergen offers a more relevant murine model of human allergic asthma and can be suitable for characterizing different intervention strategies to prevent or treat allergen-induced lung constriction.

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